

P-10 Preliminary Experiments with Corn Fiber

Preliminary Experiments with Pretreated **Corn Fiber**
Tammy **Kay** Hayward
April 5, 1995

Experiment Run Date: January **1995**

Oral Presentation: February 1995

AMOCO CRADA Bench Scale **Research Director:** Christos Hatzis

Objectives:

To **study** the effect of autoclaving on pretreated corn fiber (ECF) SSFs. To test the effect of using **lime** or ammonium hydroxide to neutralize the PCF. To study the kinetics of this new substrate and new organism (parent strain Labatt 1400). Finally, look at a materials balance with the spent solids liquids.

Materials and Methods:

PCF: The substrate **used** in this experiment **was** the pretreated corn fiber sent to NREL by AMOCO in December **of** 1994. Material from bucket **#11** **was** stirred **and** then neutralized with either lime or ammonium hydroxide. **PCF** slurry was adjusted to pH **5** prior to autoclaving. Overliming **was** not performed. **All flasks and** bubble traps were autoclaved prior to the experiment. Except for the case of the fresh, unautoclaved PCF, the other **SSF** ingredients were sterilized. A 40 % w/w PCF concentration **was** used. **This** concentration was chosen based on tests with the continuous pump in hope that data from these **flasks** could be used to design continuous **SSF** experiments.

CSL: The nutrient source employed was 1% v/v Grain Products Corporation Corn Steep Liquor. This CSL is a very thick mixture containing solids. Filter sterilization of the raw CSL proved difficult. **So,** a 10% dilution of the CSL in DI water **was** adjusted to pH **5** with ammonium hydroxide, and autoclaved for 30 minutes. This autoclaved stock solution was used in the **SSF flasks**.

Cellulase: The PDU lot of CPN was used **as** the cellulase enzyme. A 10x dilution in D.I. water was filter sterilized and employed at **2.55 mL** per total 100 gram slurry in each 250 mL **flask**. The activity of the filtered, undiluted enzyme, **as** measured by **Bill Adney** was 70 FPU per mL. Based on the chemical analysis available at the time of the experiment, a loading of 10 **FPU** per gram of cellulose was attempted.

Yeast: The organism used in this experiment **was** from **a** plate given to NREL by **Ray Bigelis** (AMOCO) in December of 1994. A freeze back of this culture was performed. The vials were stored in the new -75 C freezer. A two stage YPD (1% yeast extract, 2% peptone, 2% dextrose) inoculum grown at **38°C** was prepared from a vial of the parent strain Labatt 1400. A 10% v/v inoculum was then used to start the **SSFs**. No adaptation to the pretreated corn **fiber** was **performed**.

Conditions: SSFs were run at 38°C and 150 rpm with bubble traps. **250 mL flasks** with 100 grams total weight. The slurry was autoclaved in the **flasks** for 30 minutes at 121°C.

Experimental Design:

The experiment was performed in duplicate as follows:

Flasks 1 & 2 Unautoclaved PCF neutralized with ammonium hydroxide

Flasks 3 & 4 Autoclaved PCF neutralized with ammonium hydroxide

Flasks 5 & 6 Autoclaved PCF neutralized with calcium hydroxide

Results:

Effect of autoclaving: Fresh, non-autoclaved PCF flasks were lighter in color (yellow-green) than their autoclaved counterparts (brown-yellow). The darker color may have been due to the formation of reaction products and/or darkening of xylose during autoclaving. Despite the visual difference between the flasks, ethanol production and glucose consumption rates were identical. Thus, autoclaving pH 5, 40% w/w PCF would not significantly alter the six carbon SSF kinetics and could be employed in the continuous system, See figure 1 “Effect of Autoclaving” for graphic comparison.

Effect of calcium and ammonium hydroxide: Neutralization of PCF is dramatically easier with ammonium hydroxide (28-30% NH_4OH) than calcium hydroxide. The calcium hydroxide is the main component in industrial lime. This powder balls up in the PCF slurry and forms hard chunks which have to be worked into the mixture. The PCF neutralized with calcium hydroxide was lighter in color than its counterpart. Nitrogen in the ammonium hydroxide may have bound to sugars in the PCF forming the darker color. Autoclaved PCF neutralized with calcium hydroxide did slightly better than those neutralized with ammonium hydroxide. Overall, however, there was not enough data to prove calcium hydroxide performed any better in SSF than ammonium hydroxide. Historically lime (calcium hydroxide based) has been used to neutralize dilute acid pretreated materials, for this reason, it was decided to use calcium hydroxide in the continuous system. See figure 2 “Effect of Calcium and Ammonium Hydroxide”.

Basic Kinetics of SSF on PCF: During this experiment, samples were taken every two hours for the first 10 hours and then 1-2 times per day. Free, monomeric glucose as measured on the YSI started around 8 g/L and dropped to 0.5 g/L in 10 hours. Ethanol continued to climb after the free glucose was consumed suggesting standard SSF consumption of cellulose. See figure 3 “Pretreated Corn Fiber SSF Kinetics”.

Contamination: At the end of this experiment, seven days (168 hours) the flasks were observed under the microscope. Long bacterial rods, diplococci and the original Labatt 1400 yeast were present. All flasks had the same degree of contamination. The GPC CSL is quite dirty. These organisms may have originated from the CSL. Any spore forming organisms may have survived the autoclaving. These dead bodies confuse microscopic observations. Their presence also sheds doubt on the relevance of the quality of organic acids and other microbial products to non-contaminated SSFs with PCF.

End point Solids and Liquors Analysis: Also at this time of seven days, the flasks were harvested. Liquids were filter sterilized and given to the CAT task for analysis. There was very little insoluble solids remaining, so solids from all six flasks were combined, autoclaved, filtered, washed and also sent to the CAT task. See Appendix 1 for the CAT task reports and chemical analysis summary. A 10 mL slurry sample was pelleted in a centrifuge and washed twice for each of the flasks in order to determine the insoluble solids concentration at time final.

SSF Material Balance Assumptions: Since this SSF experiment was not initially designed to close a materials balance, the following assumptions were made:

1. Instead of sacrificing a **flask** at time zero, **it** is assumed that the composition of the **flask** can be determined since we know the composition **and** amount of each ingredient. **This** is a relatively safe assumption.
2. The cell mass **was** not measured at any time during the experiment. The initial and final cell concentration is assumed based on previous **SSF** experimental data with **paper** and D₅A. This assumption **is** more risky, although the amount of carbon in the cells during **SSF** is relatively small.
3. Although the insoluble solids were measured at time final, they were not measured at time zero.
4. Carbon dioxide is calculated by stoichiometry based on fermentation products.

Excel Material Balance Spreadsheet: The **SSF** material balance spreadsheet used previously in the CRADA for paper substrates was significantly modified by Christos Hatzis to examine corn fiber **SSFs**. The spreadsheet performs balances on each of the following components: cellobiose, glucose, galactose, mannose, xylose, arabinose, lignin, ethanol, cell mass, carbon dioxide, glycerol, acetic acid, lactic acid and succinic acid. The spreadsheet is also divided into carbon in at time zero, and carbon out at time final. **Each** of those are **further** divided into contributions made by the solid and liquid portions. There is also a conversion column and a yield column, a total carbon recovery and a list of yields. See figure 4 “40% Pretreated Corn Fiber **SSFs**”.

Distribution of Carbon at Time 0: At the beginning of the **SSF**, 1/3 of the carbon is glucose (some monomer, some oligomer, some polymer), 1/3 in form of five carbon sugars (**again** some polymer), 22% in a form that analyzes as lignin (this may include protein and extractives) **and** the remainder **as** ethanol from the inoculum, acetic acid from pretreatment and other **six** carbon sugars. See figure 5 “Carbon In”

Distribution of Carbon at Time Final: After seven days of **SSF** the distribution of carbon has changed significantly. Ethanol product forms **18%** of the total carbon, by-products (mostly carbon dioxide) form another **18%**, the five carbon sugars are still approximately 1/3, 24% of the carbon analyzes as lignin, 12% remains **as** unconverted six carbon sugars. See figure 6 “Carbon Out” A graphical representation of change in the composition of the material after **SSF** is also available. See figure 7 “Carbon Distribution in **Solids** and Liquors”.

Lignin: The **lignin** balance closed within 2.86%, with the excess on the outlet side. **Lignin** is found in both the liquid and the solid portions. The corn fiber is known to be high in protein. The portion of the feedstock that analyzes **as** **lignin** would probably include the protein. **Since** **lignin** is conserved, it may be that the protein is conserved **as** well in **SSF** (conservation is overall, additions are made by the enzyme and yeast cells). Also corn oils which are visible during **SSF** are not accounted for on the spreadsheet and **will** therefore add to error.

Six Carbon Sugars: Glucose, galactose and mannose are consumed in the **SSF**. Conversion of the sugars breaks down **as** follows: 81.8% of the glucose, 60% of the mannose and 13.88% of the galactose. It seems that **Labatt 1400** prefers to consume glucose **and** mannose over galactose. This preference is common with other yeast. Six carbon sugars in the **solids** are successfully liquefied by the cellulase enzyme complex. Cellulose conversion efficiency is 96.5%.

Five Carbon Sugars: Xylose and arabinose balances only close within 18 and 15% suggesting conversion by the contaminant bacteria or significant errors in their measurement. Also the percentage of total five carbon sugars in the solids decreases over the course of the **SSF**, suggesting a xylanase activity in the CPN cellulase preparation. The breakdown of the cellulose in this substrate may also lead naturally to the release of five carbon sugars from the solids or there may be bona fide xylanase activity.

Oligomeric Sugars: There is a considerable amount of oligomeric sugars left in the liquor at the end of the **SSF**. For example, the **YSI** read 0.1 to 0.8 g/L glucose at time zero. The **CAT** task measured anywhere between 0.69 and 1.35 g/L in the six flasks at time zero. After a 4% acid hydrolysis the glucose level jumps to between 5.58 and 6.5 g/L. The oligomeric glucose after **SSF** is the difference of the above, between 5.9 and 4.2 g/L. Some of this oligomeric sugar is probably sucrose from the CPN enzyme preparation, (the enzyme is suspended in around 300 g/L sucrose). These oligomeric sugars in the liquid go unused for ethanol production and contribute to low yields. There is also a significant amount of oligomeric xylose and arabinose.

Cellulase Loading: The cellulase enzyme loading can be back calculated based on the insoluble cellulose number as follows: 35 g/L insoluble solids at time zero, times 44.25% leaves 15.49 g/L cellulose. Each **SSF** flask had a working weight of 100 grams so 1.549 g were cellulose. The CPN enzyme was diluted ten fold (7 FPU/mL activity after dilution) and then 2.55 mL of it were added to each flask. The resulting enzyme loading is 11.52 FPU/ g of cellulose.

Ethanol Yield: The ethanol process yield is 60.8% based on six carbon sugars. The low yield reflects the amount of unconverted oligomeric sugars in the liquid and left over polymer in the solids. The ethanol metabolic yield is 82.9% based on consumed six carbon sugars. This reflects on the Labatt 1400 and the contaminant bacteria. Product distribution on a gram of product per 100 grams of consumed glucose demonstrated considerable amounts of by-products, 6% to cell mass, 5% to succinic acid (**HPLC**) 3% to Lactic acid, 2% to glycerol, and 1% to acetic acid. See figure 8 "Product Distribution".

Cellulase Efficiency: The cellulose in this substrate is more easily broken down into glucose than any substrate tested so far. The release of glucose polymer from the solid portion of the substrate is 96.5% according to the Excel spreadsheet. This conversion number is based on the chemical analysis of the washed solids before and after **SSF** as well as the washed solids concentrations in those streams.

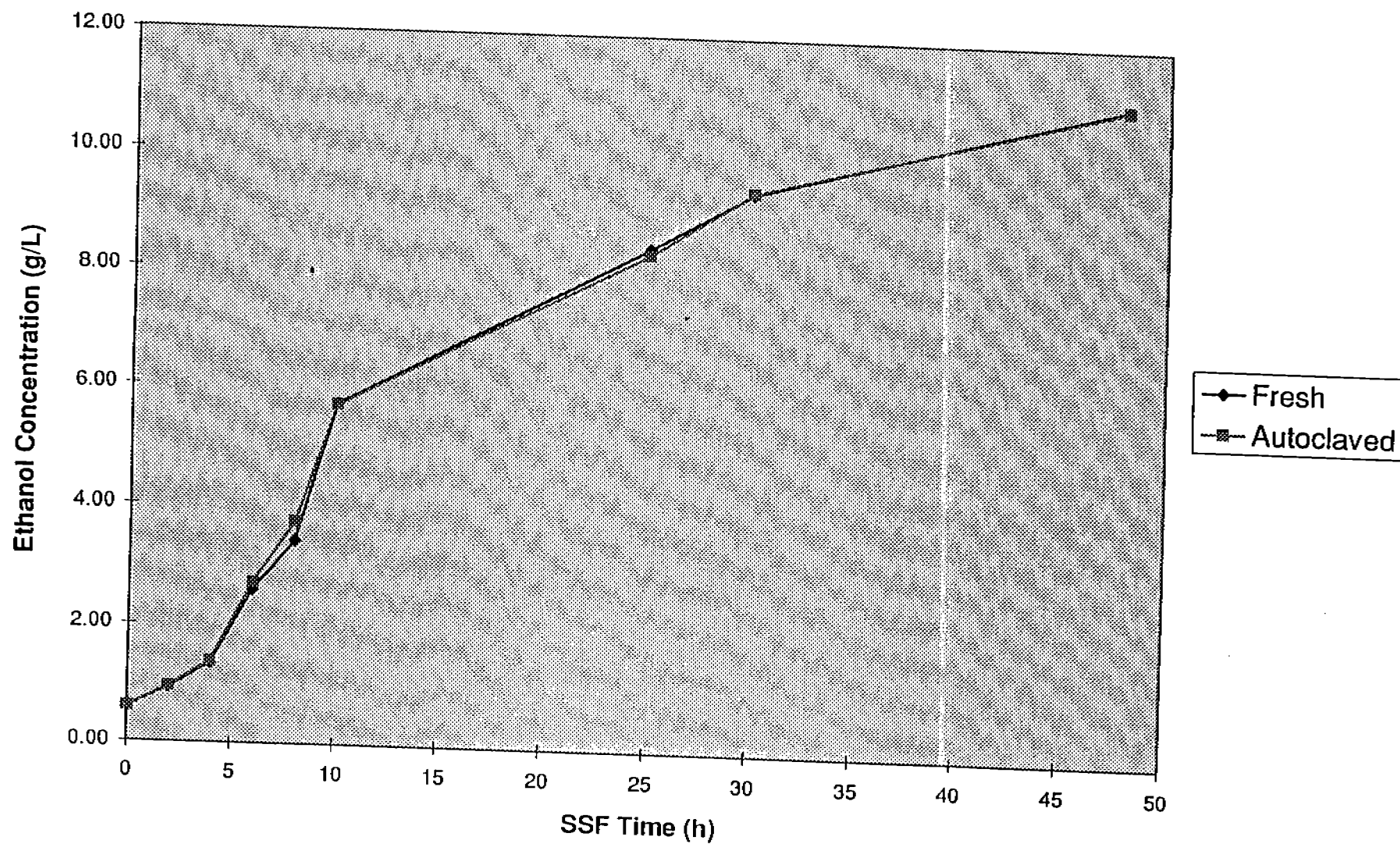
Conclusions:

1. Autoclaving pretreated corn fiber did not have an effect on the rates of glucose consumption or ethanol yield from six carbon sugars.
2. There is a slight increase in **SSF** yields with calcium hydroxide, the main ingredient in lime, over ammonium hydroxide. For economic and historical reasons, calcium hydroxide is the preferred method.
3. The kinetics of **SSF** with pretreated corn fiber are special due to the concentration of free glucose at the beginning of the reaction and the relatively fast enzymatic release of glucose from cellulose. The measured concentration of glucose in the liquor of the **SSF** drops from 8 g/L to 0.2 g/L in 10 hours.

4. This experiment **was** used **as** the first test for the SSFExcel Carbon Balance **with pretreated corn** fiber. The carbon recovery overall was **96.26%**. Cellulose conversion **was** 96.5%. This makes pretreated corn fiber the most digestible substrate I have ever tested. Ethanol process yield however **was** low at 60.8%. The low yield is **due primarily** to unconverted six **carbon** oligomers in the liquor, Although **this** substrate **is high** in oils **and** protein, these have not yet been incorporated into **the** spreadsheet. A third of this substrate is five carbon **sugars which** makes the conversion of this portion critical to its economics.

Figure 1

Effect of Autoclaving



Effect of Calcium or Ammonium Hydroxide for Neutralization
of Pretreated Corn Fiber

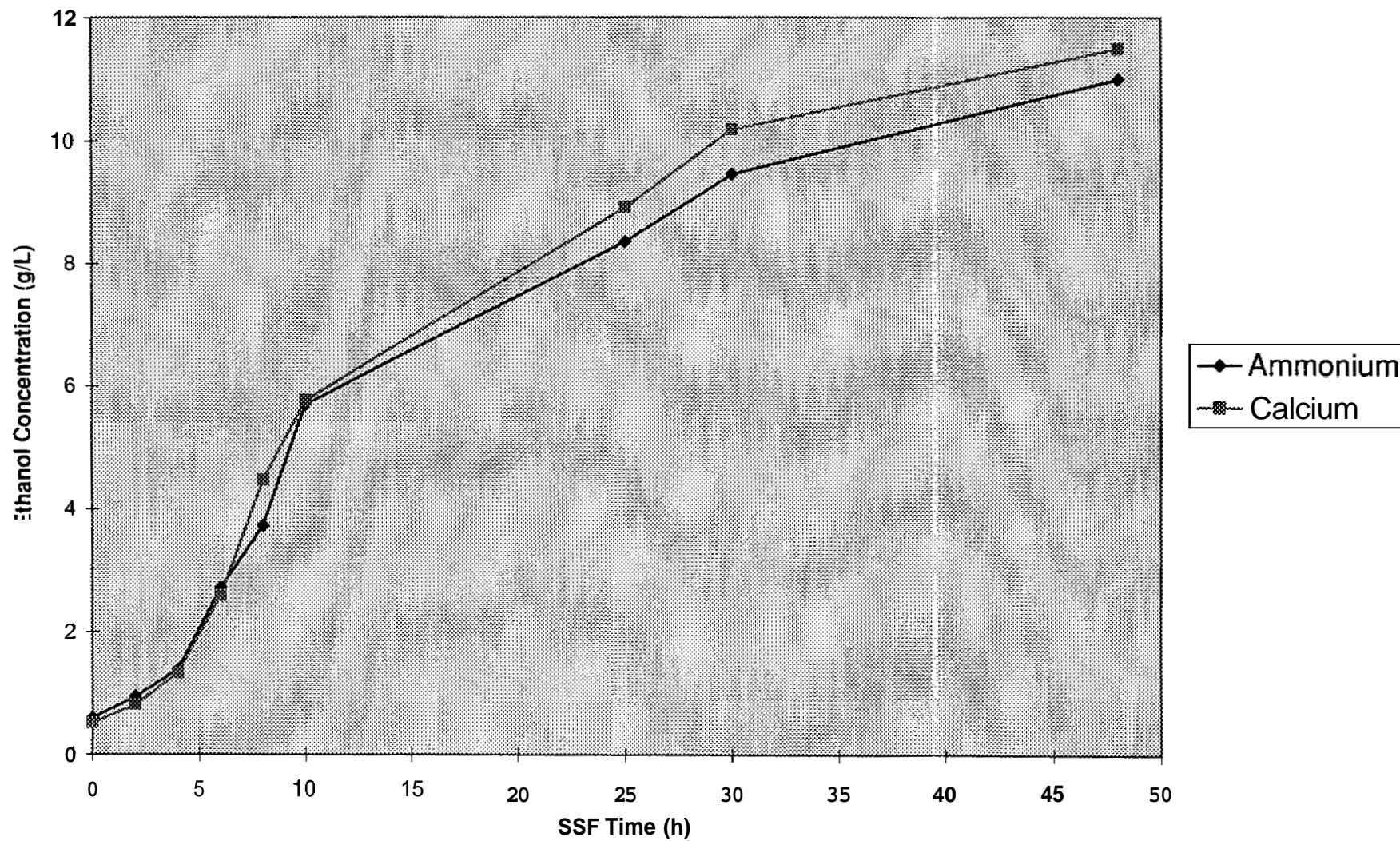
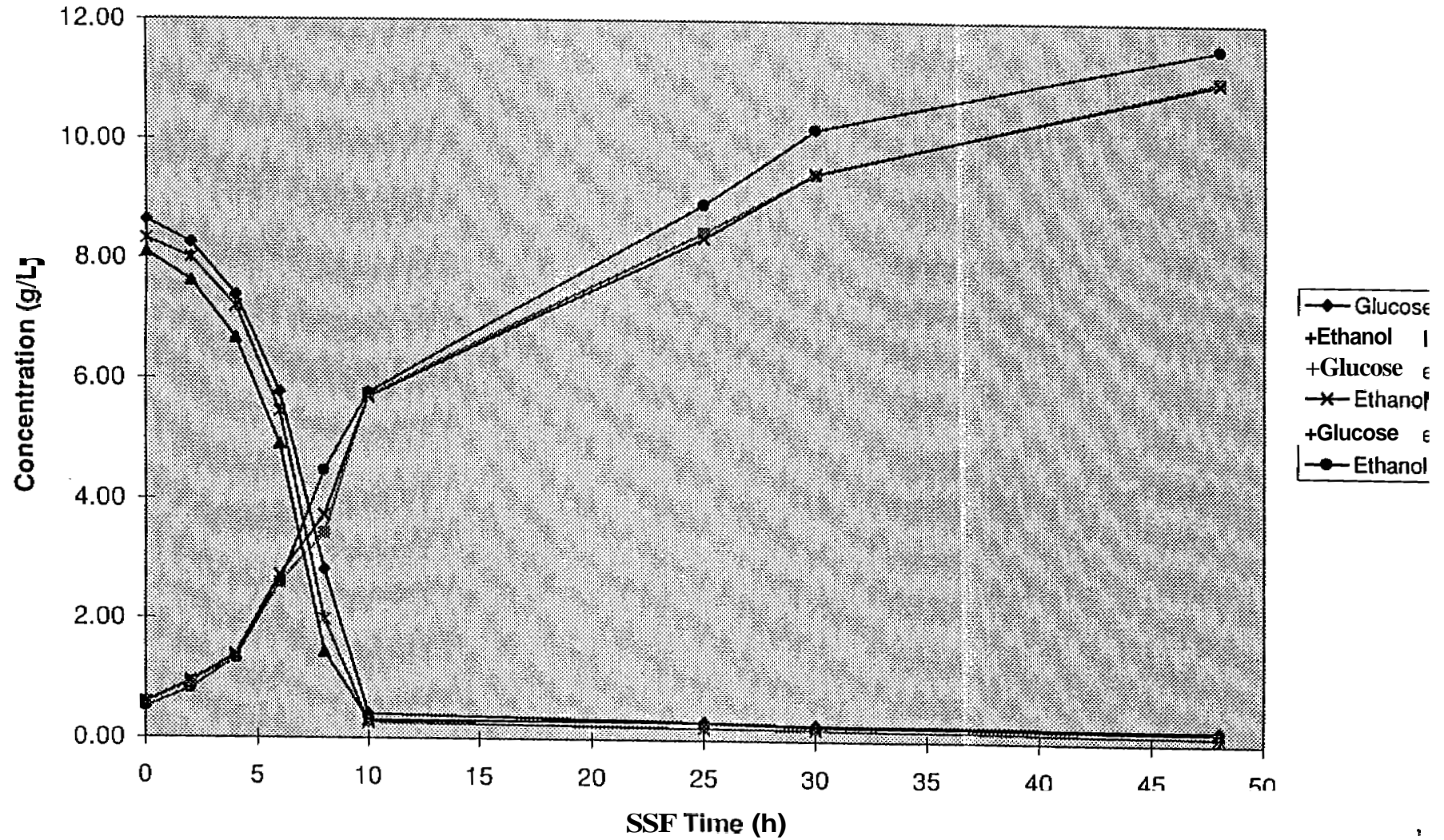


Figure 3

Pretreated Corn Fiber Kinetics



SSF CARBON BALANCE: 40% Pretreated Corn Fiber

Sample: ECF Experiment 1

Pretreatment:

Run:

SOLIDS BALANCE	In	Out
Lignin (%):	30.83	79.61
Insoluble Solids (%):	3.50	1.40

Cellulose Conversion:	96.5%
Overall C6 Sugar Conversion:	73.3%
Overall C5 Sugar Conversion:	17.2%
Ethanol Process Yield (% Theor):	60.8%
Ethanol Metabolic Yield (% Theor):	82.9%

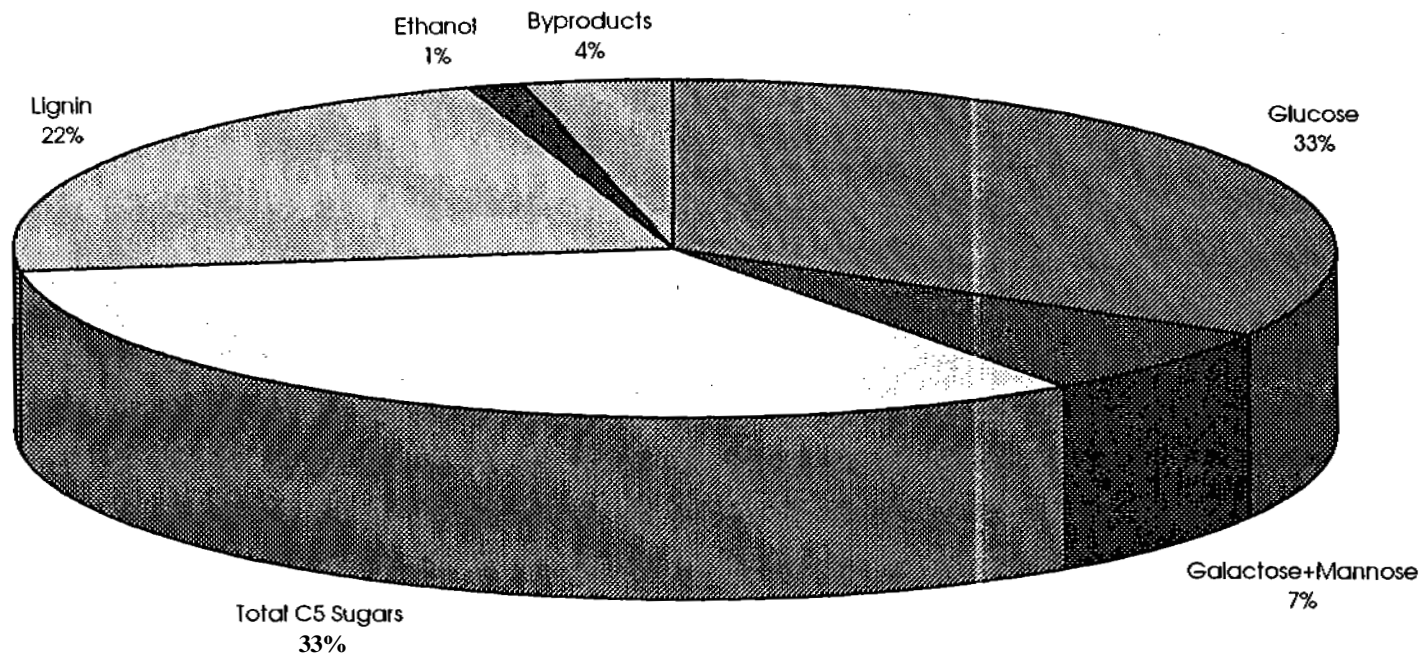
Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion (In-Out)/In (%)	Yield g product/ 100 g C6 con
	In Solids			In Liquor			Total	In Solids			In Liquor			Total		
	(% dry wt)	(C-mole/Kg Sh	(% Total In)	(g/L)	(C-mole/Kg Sh	(% Total In)		(C-mole/Kg Sh)	(% dry wt)	(C-mole/Kg Sh	(% Total Out)	(g/L)	(C-mole/Kg Sh			
Cellulose				0.00	0.000		0.000				0.00	0.000		0.000		
Glucose	51.27	0.598	50.7	18.05	0.580	49.3	1.178	4.42	0.021	9.6	5.90	0.194	90.4	0.214	01.81	
Galactose	1.42	0.017	11.1	4.12	0.133	88.9	0.149	0.54	0.003	2.0	3.63	0.126	98.0	0.128	13.88	
Mannose	0.12	0.001	1.7	2.55	0.082	98.3	0.083	0.00	0.000	0.0	1.02	0.033	100.0	0.033	60.04	
Xylose	9.02	0.105	14.7	19.04	0.612	85.3	0.717	2.43	0.011	1.9	17.55	0.576	98.1	0.588	16.07	
Arabinose	4.54	0.053	12.2	11.80	0.379	87.8	0.432	0.55	0.003	0.7	11.01	0.362	99.3	0.364	15.72	
Lignin	30.83	0.516	65.4	5.93	0.273	34.6	0.789	79.61	0.533	65.6	5.92	0.279	54.4	0.812	-2.06	
Ethanol				1.00	0.042		0.062				14.33	0.613		0.613		42.39
Cell Mass				0.20	0.008		0.008				2.00	0.079		0.079		5.73
Carbon Dioxide												0.313		0.313		44.39
Glycerol				0.08	0.002		0.002				0.84	0.027		0.027		2.44
Acetic Acid				1.61	0.052		0.052				1.45	0.061		0.061		0.86
Lactic Acid				0.49	0.016		0.016				1.50	0.049		0.049		3.22
Succinic Acid				1.61	0.053		0.053				3.22	0.108		0.108		5.23
Total	90.29	1.209	36.6		2.232	63.4	3.521	86.71	0.570	16.8		2.020	83.2	3.389		104.25

C6 SUGAR OVERYIELD	94.26%
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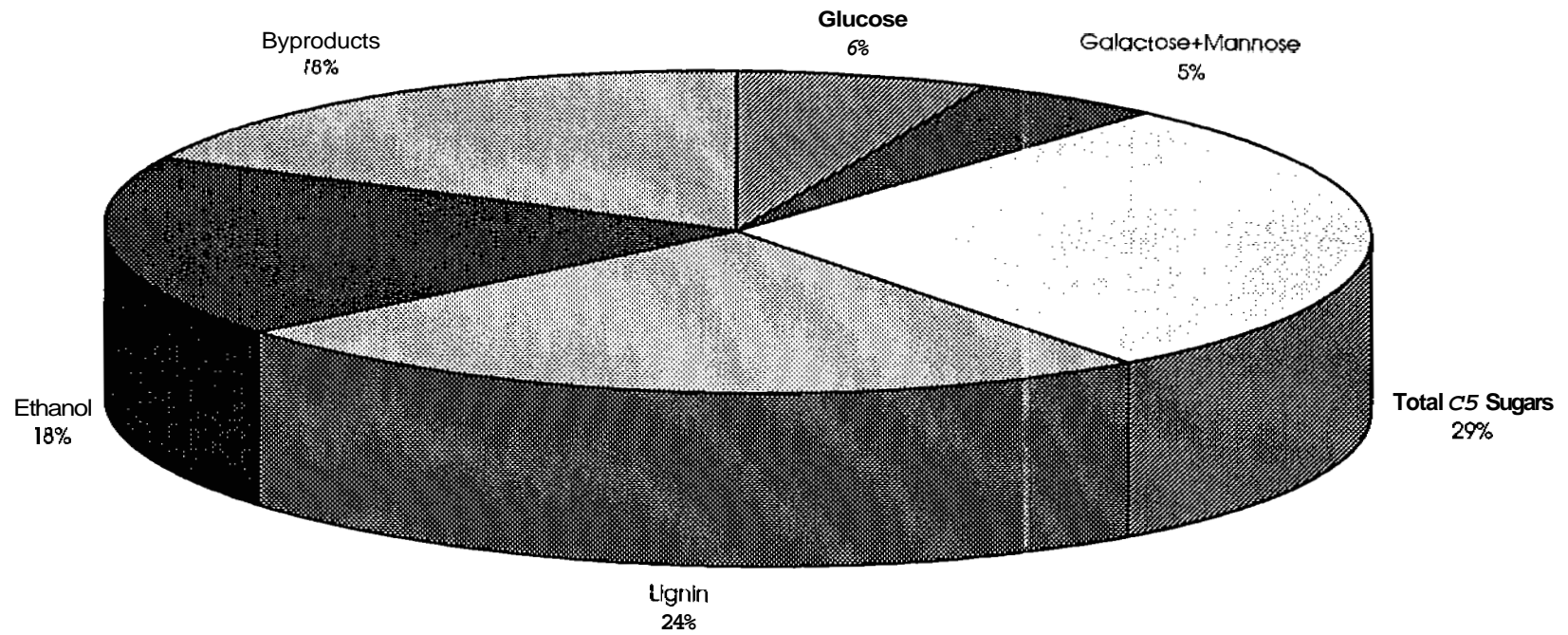
Carbon In

Distribution of Carbon in SSF
Carbon In

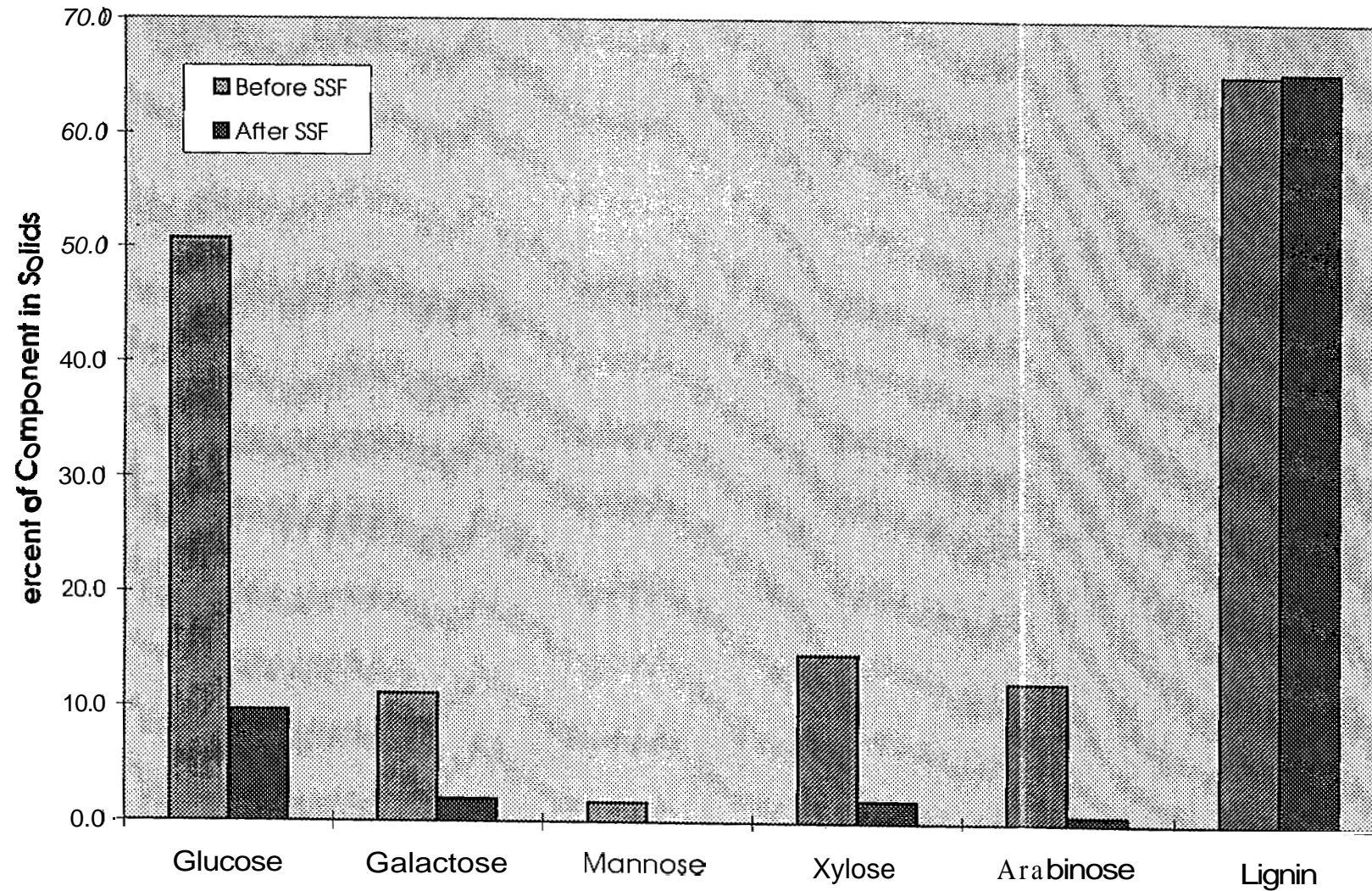


Distribution of Carbon in SSF

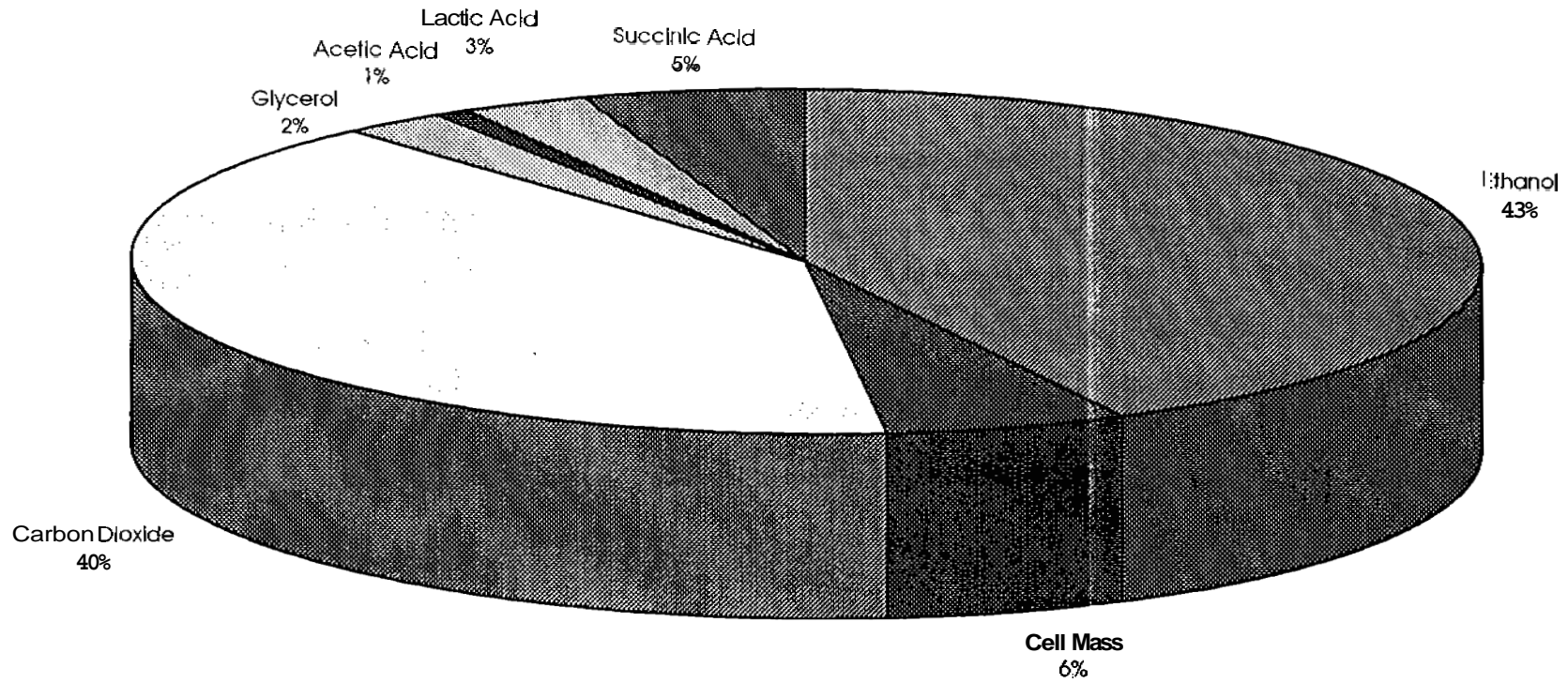
Carbon Out



Carbon Distribution In Solids and Liquor in SSF



Product Distribution
(g product / 100 g glucose consumed)



CARGILL CORN FIBER						
	Untreated ¹					
	Whole slurry ²			Washed solids ³		Liquor
	(% Dry weight)	(% Dry weight)	(% Wet weight)	(% Dry weight) 61.5C		Monomeric oligo (g/L) Total (g/L)
Glucose	30.29	33.75	7.62	51.27		19.03 26.10 45.13
Galactose	3.68	3.97	0.90	1.42		6.73 3.58 10.31
Mannose	0.54	0.00	0.00	0.12		3.80 2.58 6.38
Xylose	30.29	24.00	5.42	9.02		25.50 47.61
Arabinose	13.17	14.86	3.35	4.54		22.13 29.50
Lignin						
Klason	3.83	9.40	2.12	24.83		
Acid Soluble	6.76	7.10	1.60	6.00		14.82
Extractives ⁴	12.41					
Ash	0.70	0.94	0.21	0.29		
Other	6.73	5.98		9.42		
Total	100.00	100.00	21.22	100.00		

Starch	15.64	15.74	3.5	1.89		
Cellulose ⁵	11.42	14.64	3.30	11.25		
C	40.53	44.73	10.10	50.62		
H	5.65	6.52	1.47	6.11		
N	1.62	1.46	0.33	2.34		
Protein ⁶	10.13	9.09	2.05	14.63		
Total Solids			21.48			
Furfural						0.320
HMF						0.061
Acetic Acid						4.030

1. The sample was extracted with 95% EtOH, lyophilized, and then analyzed according to standard CAT protocols
2. The whole pretreated slurry was lyophilized and then analyzed according to standard CAT protocols
3. The solids were separated from the pretreated slurry and washed twice with water, according to the CAT protocol. and then analyzed according to standard CAT protocols
4. Extractives after extraction with 95% EtOH
5. Cellulose content was determined from the measured total glucan and starch contents
6. Protein was determined based on percent nitrogen

5.13
10.31
6.31
= 21.75

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis
No.
95-021Page
1 of 1

Project Title: Extruded Corn Fiber SSFs (ECF1); Work Package ET60

NREL In-House



Current Subcontractor



CRADA



Other



Date Samples Delivered: January 25, 1995

Date Work Promised: n/a

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: February 28, 1995

NREL Notebook: #1638 p 017-036, #1385 p 028

Estimated Hours Required: Not Given

Sample description: Filtered SSF Liquids

Actual Hours Spent: 144

Summary of Requested Work: sugars pre- and post- 4% acid hydrolysis, levulinic acid, glycerol, lactic acid, acetic acid, HMF, furfural, ethanol.

Proposed Approach: Standard CAT task analytical methods, standard LAP's augmented to measure levulinic acid by in-house analysts.

Work Required: Sample Prep ☒ NDF/ADF ☐ Acid Digest ☒ HPLC ☒ YSI ☒ GC ☒ Other: pH

Results and Comments ☐ % As Received 3.83 ☐ % Dry Weight ☒ Other: mg/mL

Sample	pH	CEL	G	X	GA	A	M	SA	LAC	GLY	AC	HMF	FL	LEV	EtOH	YSI-G
1 Flask #1 ECF1 (00602) as received	ave 4.82	nd	1.35	9.59	2.61	9.55	nd	3.05	1.49	0.88	1.75	nd	nd	nd	13.6	0.96
sd	--	--	0.05	0.12	0.07	0.19	nd	0.01	0.00	0.00	0.01	--	--	--	--	0.01
Flask #1 ECF1 (00602) after 4% hydrolysis	ave --	nd	6.54	16.67	3.88	10.41	0.84	--	--	--	--	--	--	--	--	6.95
sd	--	--	0.01	0.19	0.14	0.11	0.02	--	--	--	--	--	--	--	--	0.11
Flask #2 ECF1 (00603) as received	ave 4.72	nd	1.29	9.90	2.73	9.54	nd	2.91	1.50	0.79	1.88	nd	nd	nd	14.4	1.07
sd	--	--	0.04	0.04	0.03	0.17	nd	0.00	0.00	0.00	0.20	--	--	--	--	0.04
Flask #2 ECF1 (00603) after 4% hydrolysis	ave --	nd	6.31	19.24	4.51	12.42	1.32	--	--	--	--	--	--	--	--	6.51
sd	--	--	0.34	0.01	0.01	0.16	0.17	--	--	--	--	--	--	--	--	0.19
3 Flask #3 ECF1 (00604) as received	ave 4.87	nd	0.69	9.90	2.73	9.54	nd	2.75	1.50	0.83	1.71	nd	nd	nd	14.1	0.61
sd	--	--	0.04	0.04	0.03	0.17	nd	0.00	0.00	0.00	0.00	--	--	--	--	0.00
Flask #3 ECF1 (00604) after 4% hydrolysis	ave --	nd	5.61	17.42	4.07	10.82	1.13	--	--	--	--	--	--	--	--	5.81
sd	--	--	0.08	0.40	0.07	0.29	0.10	--	--	--	--	--	--	--	--	0.20
4 Flask #4 ECF1 (00605) as received	ave 4.93	nd	0.85	8.88	1.96	8.65	nd	2.95	1.48	0.88	1.79	nd	nd	nd	14.9	0.70
sd	--	--	0.01	0.01	0.02	0.05	nd	0.00	0.00	0.00	0.01	--	--	--	--	0.01
Flask #4 ECF1 (00605) after 4% hydrolysis	ave --	nd	5.58	16.82	3.50	10.39	0.98	--	--	--	--	--	--	--	--	5.81
sd	--	--	0.11	0.10	0.09	0.02	0.03	--	--	--	--	--	--	--	--	0.01
5 Flask #5 ECF1 (00606) as received	ave 4.89	nd	0.67	8.38	1.87	8.31	nd	3.97	1.50	0.85	2.01	0.02	nd	nd	14.5	0.54
sd	--	--	0.03	0.06	0.03	0.01	nd	0.00	0.00	0.00	0.00	0.00	--	--	--	0.02
Flask #5 ECF1 (00606) after 4% hydrolysis	ave --	nd	5.66	17.44	3.66	10.96	0.92	--	--	--	--	--	--	--	--	5.73
sd	--	--	0.28	0.37	0.13	0.34	0.14	--	--	--	--	--	--	--	--	0.16
6 Flask #6 ECF1 (00607) as received	ave 4.89	nd	1.00	9.01	1.77	8.86	nd	3.71	1.50	0.82	1.96	nd	nd	nd	14.5	0.88
sd	--	--	0.05	0.02	0.01	0.04	nd	0.01	0.00	0.00	0.19	--	--	--	--	0.02
Flask #6 ECF1 (00607) after 4% hydrolysis	ave --	nd	5.68	17.70	3.38	11.08	0.90	--	--	--	--	--	--	--	--	5.74
sd	--	--	0.16	0.39	0.14	0.54	0.11	--	--	--	--	--	--	--	--	0.12

binose; AC=acetate; CEL=cellulose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LAC=lactic acid; LEV=levulinic acid; M=mannose; nd=not detected; X=xylose; YSI-G=Glucose determined by YSI

Name(s) of CAT Staff Working on Project: B. Ashley, F.P. Eddy, D. Johnson, and D. Templeton

CAT Task Leader: D. Ehlman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis
NO.
95-020

Page
1

NREL In-House ☐ Current Subcontractor ☐ CRADA ☒ Other ☐

Date Samples Delivered: 2/9/95

Date Work Promised: 2/14/95

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: 2/15/95

NREL Notebook: #1561, p017, #1382, p108

Estimated Hours Required: 4

Samples from Feedstock Lot No.: n/a

Actual Hours Spent: 4

Summary of Requested Work: Complete compositional analysis, protein content.

Proposed Approach: Standard Laps by validated outside laboratory, protein content by in house CHN analysis.

Work Required: Sample Prep ☒ NDF/ADF ☐ Acid Digest ☒ HPLC ☒ YSI ☐ GC ☐ Other: ☐

Results and Comments ☐ % As Received ☐ % Dry Weight ☐ Other: 79.61

Sample TS G X GA A M LKL LAS AT

sd	0.21	0.33	0.11	0.02	0.07	0.0	0.29	0.23	0.03	
ave										
3										
ave										
sd										
4										
ave										
sd										
5										
ave										
sd										
6										
ave										
sd										
7										
ave										
sd										

A=arabinose; AC=acetate; AD=detergent ash; AT=total ash; C=mass % carbon; CE=cellulose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; H=mass % hydrogen; HC=hemicellulose; L=detergent lignin; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; N=mass % nitrogen; nd=not detected; nr=not requested; P=protein; TS=total solids; UA=uronic acids; X=xylose; *=calculated from nitrogen measured by CHN

Name(s) of CAT Staff Working on Project: Larry Brown, CAT Task Leader: Tina Ehrman

Signature: *Larry Brown* *Rory Rice*

Signature: *Tina Ehrman*